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PATENT
Docket No.: GOW-013-US (109144-143)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	I. ALTOSAAR et al.		
Serial No.	:	10/723,083	Art Unit:	To Be Assigned
Filed	:	November 26, 2003	Examiner:	To Be Assigned
For	:	**Production of GM-CSF in Plants**		

Customer No.: 28089

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I hereby certify that this correspondence is being deposited on the date below with the U.S. Postal Service as Express Mail, in an envelope bearing Express Mail No. EV 324102149 US, and addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Date: March 22, 2004

Signature: Leslie Serunian
Leslie Serunian, Reg. No. 35,353

Transmittal Letter Accompanying Certified Copy of Priority Document

Dear Sir:

Provided herewith for filing in the above-identified patent application is a certified copy of Canadian Patent Application No. 2,410,702 to which the instant application claims priority.

No fee is due for the filing of the certified copy of the priority document. However, should any fee(s) be deemed to be properly assessable during the pendancy of this application, the Commissioner is hereby authorized to charge any such fee(s), or to credit any overpayment, to Deposit Account No. 08-0219, Order No. GOW-013-US (109144-143).

Respectfully submitted,
HALE AND DORR LLP

Date: March 22, 2004

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La présente atteste que les documents ci-joints, dont la liste figure ci-dessous, sont des copies authentiques des documents déposés au Bureau des brevets.

This is to certify that the documents attached hereto and identified below are true copies of the documents on file in the Patent Office.

Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,410,702**, on November 26, 2002, by **ILLIMAR ALTOSAAR and RAVINDER SARDANA (APPLICANTS AND CO-INVENTORS)** and Anil Dudani, Peter Ganz, Eilleen Tackaberry, for "Production of Human Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in the Seeds of Transgenic Rice Plants".

Agent certificateur/Certifying Officer

March 11, 2004
Date

Canada

(CIPO 68)
04-09-02

OPIC CIPO

Title of Invention:
Production of Human Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in the Seeds of Transgenic Rice Plants.

Description of Invention:
This is a method for producing biopharmaceutical proteins in a safe and cheap method using transgenic rice. Human granulocyte macrophage colony stimulating factor (GM-CSF), a cytokine with many applications in clinical medicine, was produced specifically in the seeds of transgenic rice plants. A rice endosperm-specific glutelin promoter Gt1 was used to direct the expression in seeds. Transgenic rice (confirmed by PCR; FIG.1) seed extracts contained the recombinant human GM-CSF protein (Western blot; FIG. 2) up to a level of 1.3% of total soluble protein (Table 1). Transgenic rice seed extracts actively stimulated the growth of human TF-1 cells suggesting that the seed-produced GM-CSF is stable and biologically active. Rice seeds by way of their safe and inert endosperm cell tissue provide an economic and efficient production and delivery system for the production of useful protein agents for the human pharmaceutical market, the veterinary drugs market and industrial reagents market.

What problem does the invention solve?
This process provides human GM-CSF at significantly reduced costs when compared with other methods like extraction from human cells and blood, production in recombinant hosts like bacterial, animal, yeast or insect cells. This work has the following superior attributes.

a) cost reduction
b) reduced risk of contamination with human pathogens
c) long-term storage of human GM-CSF in rice seeds
d) yield
e) the protein produced in transgenic rice seeds is in glycosylated forms or unglycosylated form. The glycosylation pattern of this human protein may be different when produced in transgenic rice seeds as here and this attribute may contribute additional stability or activity features to the drug formulation.

Original features of this invention:

1) construct comprising of Gt1 promoter, Gt1 (glutelin1) signal sequence and mature coding sequence of human GM-CSF with additional methionine codon at the beginning of mature human GM-CSF sequence. The GM-CSF sequence at its 3' end in the construct is followed by NOS terminator sequence.
2) Directing expression of human GM-CSF in rice seeds.
3) Using a particular combination of binary vector and an *Agrobacterium* strain to transform rice.
4) Transgenic rice plants containing the above construct.
5) Japonica rice variety Xiushui 11 transformed with our genetic construct.
6) Japonica rice variety Xiushui 11 seeds transformed with our genetic construct.

Immediate and possible future uses of human GM-CSF produced in transgenic rice:
1) Research reagent
2) Use in chemotherapy
3) Use in bone marrow transplantations
4) Use in patients with AIDS

The main opportunity for transgenic rice seed-produced human GM-CSF is for the treatment of medical conditions and infections in individuals diagnosed with low numbers of granulocytes and defective formation of blood cells.

We Claim:

1. The invention as substantially described herein.

TABLE 1

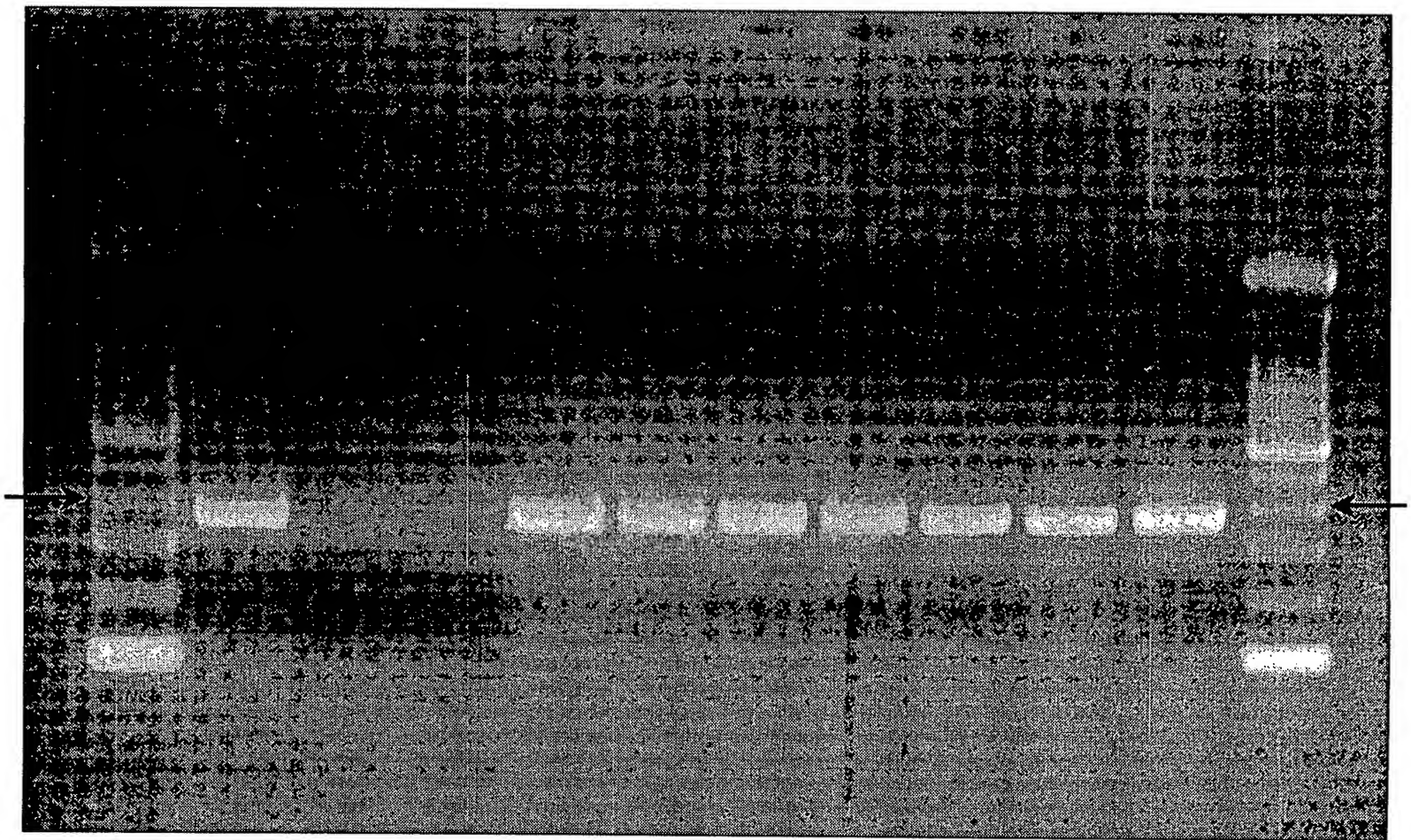
GM-CSF Levels in Protein Extracts of Seeds from Transgenic Rice Plants

PLANT	GM-CSF (μg/mL)	Total Protein (mg/mL)	% GM-SCF of Total Protein
GM-CSF 1	28	2.2	1.3
GM-CSF 5	5.6	2.3	0.24
GM-CSF 6	28	2.4	1.2

GM-CSF levels were determined by ELISA using R&D Systems Quantikine ELISA kit.

Fig. 1

PCR analysis on transformed rice DNA

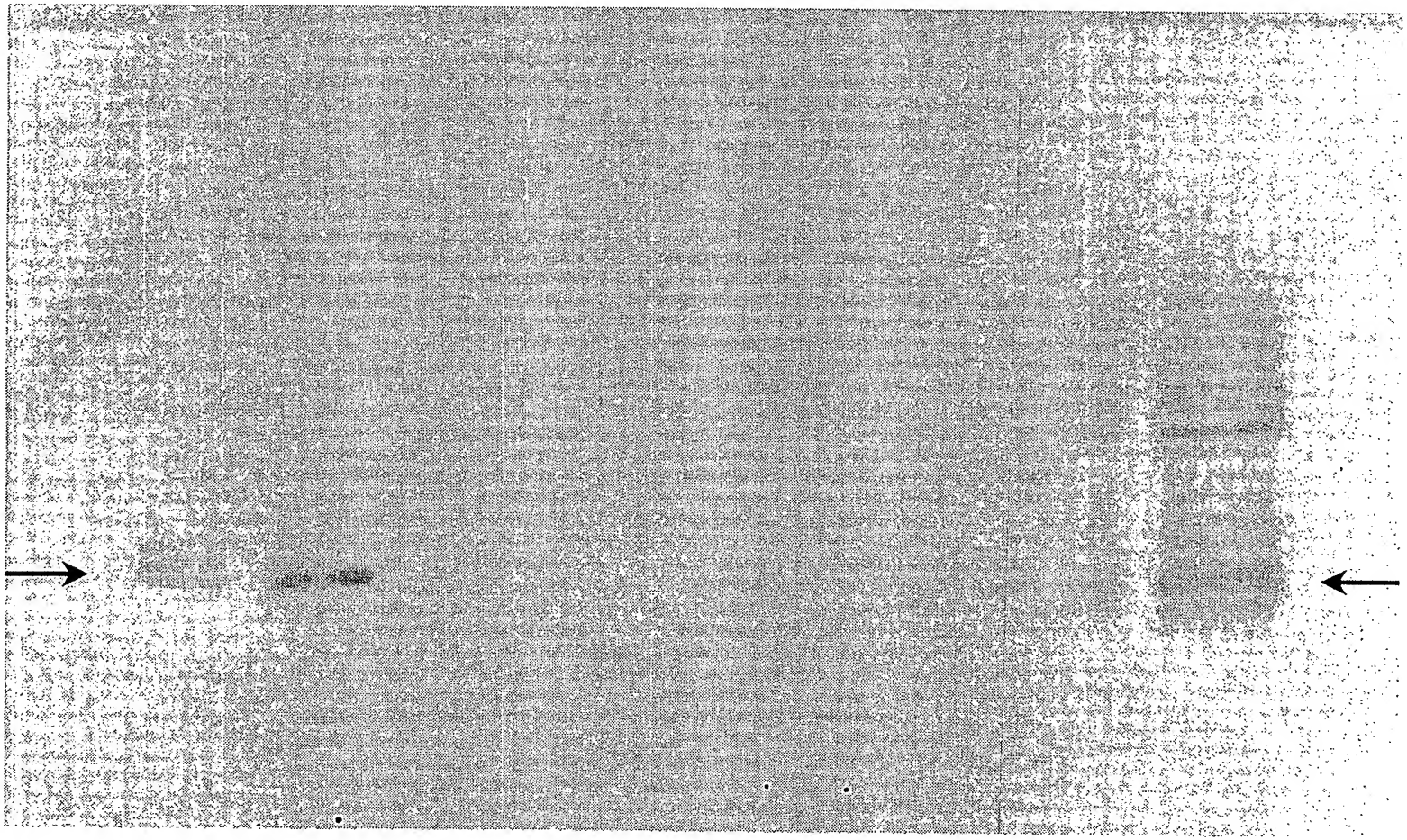


PCR = Polymerase chain reaction; NT = NON-TRANSFORMED
M = Marker.

Fig. 2

Western blot

GM-CSF commercial | NT seed extract | M | Transformed rice #1 seed extract



NT = NON-TRANSFORMED; M = MARKER